PATENT
454313-2339

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Phillipe JEANNIN
Appln. Serial No.	:	08/863,692
Filed	:	May 27, 1997
For	:	**INSECTICIDAL COMBINATION TO CONTROL MAMMAL FLEAS, IN PARTICULAR FLEAS ON CATS AND DOGS**
Examiner	:	S. Clardy
GAU	:	1616

#2 Sucp 11-2-99

745 Fifth Avenue
New York, New York 10151

DECLARATION UNDER 37 CFR 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Dr. Alan A. Marchiondo, declares and says that:

1. I am currently employed by Merial LTD, the assignee of the above-captioned application, where I am a research scientist. My responsibilities include conducting pharmaceutical and biological evaluations (basic, preclinical or clinical), and target animal safety trials for development research and registration of animal health products in regional and global markets. I am responsible for supervising the maintenance and propagation of ecto— and endoparasite colonies/cultures for *in vitro* and *in vivo* efficacy trials. I have read and am familiar with the application.

2. My education, training and experience are as follows:

As evidenced from my curriculum vitae copy attached hereto, I have extensive training and experience in the field of parasitology. Accordingly, I am considered by my peers to be an expert in the field to which the application pertains.

3. I am making this Declaration in response to comments raised in the Office Action dated March 24, 1999, including the remarks which criticized the fact that there was no comparison of the inventive composition comprising compound (A) and compound (B) against a composition comprising either compound (A) and compound (B) alone. In response to those remarks the following experiments were conducted:

4. Under my direct supervision and control, tests have now been conducted which compare the inventive long-lasting compositions comprising of fipronil and (S)-methoprene with each of the two components alone.

5. The results of these tests, as reported in the Tables, demonstrate that the inventive compositions possess surprising and unexpected synergistic ovicidal activity in contrast to the results which might have been expected by an evaluation of the individual components according to the Additive Evaluation Method.

6. Specifically, under my supervisions and control, the following *in vitro* tests, as reported in Tables 1 to 6, demonstrate that surprisingly unexpected and synergistic results are observed by the inventive compositions.

A. Egg Eclosion Test

This test determines the emergence of insect larva from an egg. Each test solution, described below, is applied to a filter paper disc. Approximately twenty-five flea eggs, which are less than twenty-four hours old, are applied to each of the discs. The discs are then incubated at an adequate temperature (approximately 23-30° C) and relative humidity (approximately 70-90% RH) to support cat flea (*Ctenocephalides felis*) development for approximately 72 hours following deposition of the flea eggs onto the filter paper discs. The

geometric mean of the flea larvae that hatched from four replicates of 25 flea eggs each at each concentration tested is presented in Table 1.

Table 1: Geometric Mean of Hatched Flea Larvae

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
CONTROL	18.25	23.25	18.00
10	20.00	17.00	ND
50	18.25	3.25	ND
10 + 10	ND	ND	10.50
50 + 50	ND	ND	1.25
10 + 50	ND	ND	2.75
50 + 10	ND	ND	2.00

ND = NOT DETERMINED

In Table 1, the amount of hatched larvae when fipronil is used alone is very similar to the control at both concentrations. Thus, one would conclude that fipronil, when used alone at these concentrations, is either inactive or slightly active and (S)-methoprene, while more active than fipronil alone at 10 ppm, killed only approximately 25% of the flea eggs (see Table 2). In contrast thereto, significant activity is observed for all the compositions containing both fipronil and (S)-methoprene.

Table 2 presents the data from Table 1 as the percent inhibition of insect larva from the egg.

Table 2: Percent Inhibition of Egg Eclosion

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
10	0	26.9	ND
50	5.2	86.0	ND
10 + 10	ND	ND	41.9 (0 + 26.9)
50 + 50	ND	ND	93.1 (5.2 + 86.0)
10 + 50	ND	ND	84.7 (0 + 86)
50 + 10	ND	ND	88.9 (5.2 + 26.9)

ND = NOT DETERMINED

The data presented in parenthesis is the percent inhibition of the active substances by the Additive Evaluation Method.

As can be seen, at 10 ppm, (S)-methoprene is about 27 times more active than fipronil, which is inactive at that amount. At 50 ppm (S)-methoprene is about 16.5 times more effective than fipronil, which only inhibits the larval emergence by 5.2%. Moreover, when these two agents are combined, clearly superior activity is obtained when the composition contains 10 ppm of (S)-methoprene is present. (see e.g. 10:10 mixture or 50:10 mixture). Such a result clearly suggests synergism at lower concentrations.

B. Larval Pupation Results

This test determines the effect an active agent has on the pupation of the larvae. After the number of hatched flea larvae have been counted after approximately 72 hours following flea egg deposition on the discs, the discs are further incubated at approximately 23-30° C and approximately 70-90% RH for approximately 14-20 days following deposition of the flea eggs onto the filter paper discs. After this period, the pupae are then separated into a container, counted and the number recorded.

The geometric mean of the pupae after 20 days is reported in Table 3.

Table 3; Geometric Mean of the Pupae – 20 Days

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
CONTROL	9.1	8.2	7.7
10	1.2	0	ND
50	0.7	0	ND
10 + 10	ND	ND	0
50 + 50	ND	ND	0
10 + 50	ND	ND	0
50 + 10	ND	ND	0

ND = NOT DETERMINED

The geometric mean number of pupae was comparable between the three control groups (range of 7.7-9.1). However, only a few flea pupae developed at the fipronil concentrations of 10 and 50 ppm as evidenced by geometric means of 1.2 and 0.7 pupae,

respectively. Likewise, no pupae develop form any of the discs treated with (S)-methoprene alone or in combination with fipronil, thus demonstrating significant larvicidal activity.

The percent of larvicidal activity based upon pupae formation is reported on Table 4.

Table 4: Percent Inhibition of Pupae Formulation

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
10	86.8	100	ND
50	92.3	100	ND
10 + 10	ND	ND	100
50 + 50	ND	ND	100
10 + 50	ND	ND	100
50 + 10	ND	ND	100

ND = NOT DETERMINED

Whereas fipronil is either inactive or slightly active against flea egg eclosion at the concentrations tested, fipronil exhibited a strong inhibitory effect against the larval stages of the flea, as evidenced by percent inhibition of pupae formations of 86.8 and 92.3% at 10 and 50 ppm, respectively. All samples containing (S)-methoprene alone or in combination exhibited 100% inhibition of pupa formation. From these data, I concluded that the inventive compositions are not demonstrating their synergistic effect at the stages where the larvae pupate due to the high level of larvicidal activity demonstrated by fipronil and (S)-methoprene alone at the concentrations evaluated.

C. This test determines the effect an active agent has on the emergence of adult fleas from pupae. After the pupae have been determined from the larvicidal test, the separated pupae are returned to the incubator. On approximately Days 29-33 following deposition of flea eggs onto the discs, the pupae are agitated twice on two separate days by vigorous shaking and exposed to CO_2 in order to stimulate adult flea emergence. On day 35 following egg deposition, the pupae are again agitated and exposed to CO_2 and frozen at approximately 0° C. Once any further development and emergence has been stopped by freezing the specimens, the number of emerged adult fleas are counted and recorded. Pupae that have

not released adult fleas were dissected. Encased adults were examined and assessed for normal development. Normal encased adults were recorded with the number of emerged adult fleas.

The geometric mean of the adult flea emergence is reported in Table 5.

Table 5: Geometric Mean of Adult Flea Emergency Plus Unemerged Adult Flea

CONC. (PPM)	FIPRONIL	(S)-METHOPRENE	COMBO
CONTROL	6.8	6.4	6.5
10	1.1	0	ND
50	0.6	0	ND
10 + 10	ND	ND	0
50 + 50	ND	ND	0
10 + 50	ND	ND	0
50 + 10	ND	ND	0

ND = NOT DETERMINED

The geometric mean number of adult fleas was similar in the three control groups ranging from 6.4-6.8 emerged adult cat fleas. In the fipronil group, the number of emerged adult fleas was very similar to the number of pupae indicating no effect of fipronil on adult emergence at the concentrations evaluated. Likewise, no adult cat fleas emerged form any discs treated with (S)-methoprene or the invention compositions because no pupae were formed.

The percent inhibition of adult flea development and emergence is reported in Table 6.

Table 6: The Percent Inhibition of Adult Flea Development and Emergence

CONC. (PPM)	FIPRONIL	METHOPRENE	COMBO
10	83.8	100	ND
50	91.2	100	ND
10 + 10	ND	ND	100
50 + 50	ND	ND	100
10 + 50	ND	ND	100
50 + 10	ND	ND	100

ND = NOT DETERMINED

From these data I conclude that the invention compositions do not demonstrate a synergistic effect at the stage where the adult fleas emerges from or develops within the pupal case because of the significant levels of initial ovicidal and subsequent larvicidal activities.

7. As is apparent from the reported results, these experiments demonstrate that surprisingly unexpected results are obtained at the egg hatching stage when relatively low concentrations of (S)-methoprene are combined with fipronil. The data demonstrate that the inventive combinations possess superior and synergistic activity at the stage the larvae hatches from the flea egg. As the prior art relied upon in the rejection does not suggest this result, it is clearly unexpected. Moreover, in view of the unexpected results obtained from (S)-methoprene, one could conclude that such results would also be obtained with other compounds which exert their activity mimetics of the juvenile hormone.

8. Under my supervision and control the following *in vivo* tests were conducted upon cats and dogs in order to demonstrate the surprisingly unexpected results. Specifically, these results demonstrate that the inventive combination inhibited larvae hatching from the eggs for a surprisingly long duration when the inventive combination of active agents applied to cats and dogs.

A. Ovicidal Inhibition Test in *Ctenocephalides Felis*

This test determines the effect that a flea composition has on the inhibition of larvae hatching from flea eggs and on the inhibition of adult cat flea emergence when the composition is applied to the skin of cats infested with newly emerged adult cat fleas (*Ctenocephalides felis*). Thirty-two (16 male and 16 female) domestic shorthaired cats approximately 6-12 months old and weighing 2.35-5.66 kg were selected and housed in individual cages. On Day –12 each cat was infested with approximately 200 adult cat fleas. On Day –11 the cats were combed to remove and count the fleas and they were re-infested with approximately 200 adult fleas. At approximately 72 hours post-infestation, a procedure for collection of flea eggs was begun. Eggs were collected over approximately a 24-hour period. On Day –7 two aliquots of approximately 100 eggs each were formed from the eggs collected from each animal. One of these aliquots was

incubated at approximately 23-30° C and 70-90% RH for approximately 72 hours to determine larval hatch. The other aliquot was incubated under the same conditions for 35 days to determine the number of adult fleas that developed. Eight replicates of four animals were formed based on body weight within sex. One cat in each replicate was randomly allocated to each of four treatment groups: 1) untreated control; 2) fipronil 10% w/v solution; 3) (S)-methoprene 12% w/v solution; and 4) fipronil 10% w/v and (S)-methoprene 12% w/v combination solution. Treatments of the flea compositions were applied once topically on Day 0 at the rate of 0.5 ml/cat. On days 1, 22, 29, 26, 43, 50 and 57 each cat was infested with approximately 200 adult fleas. Eggs were collected over approximately a 24-hour period beginning three days after infestation. One aliquot of up to approximately 100 eggs, if available, from each animal at each infestation time was incubated for three days to determine larval hatch and the other aliquot incubated for 35 days to determine the number of adults that developed. The results of this trial are reported in Table 7 (Cat Dose Confirmation Trial – Percentage of Larvae That Hatched) and Table 8 (Cat Dose Confirmation Trial – Percentage of Adult Fleas That Develop).

TABLE 7
Methoprene Dose Trial in Cats Percentage [A] Larvae that Hatch

Infestation Day [B]	Untreated Control	Fipronil 10 % w/v	Methoprene 12 % w/v	Fipronil (10% w/v) + Methoprene (12% w/v)
Pretreatment	34.8	28.3	36.5	36.9
Day 1	50.6	-	0	-
% Reduction		-	100	-
Day 22	39.8	-	0	-
% Reduction		-	100	-
Day 29	42.6	0 [C]	0.3	0 [D]
% Reduction		100	99.4	100
Day 36	34.6	42.5 [E]	5.2	1.4 [F]
% Reduction		0	85.0	95.9
Day 43	55.9	58.6	4.6	3.6
% Reduction		0	91.7	93.6
Day 50	39.2	54.9	18.9	8.7
% Reduction		0	51.6	77.9
Day 57	53.8	46.0	50.4	27.3
% Reduction		14.4	6.3	49.2

[A] Retransformed mean of radians; based on the transformation are sine √(number of adults/number of eggs).
[B] Eggs were collected for 24 hours starting 72 hours after infestation. Approximately 100 eggs were incubated for 35 days.
[C] One animal with a few eggs; no adults developed.
[D] Three animals with eggs; no adults developed.
[E] Five animals with eggs incubated.
[F] Six animals with eggs incubated

TABLE 8
Methoprene Dose Confirmation Trial in Cats Percentage [A] of Adults that Develop

Infestation Day [B]	Untreated Control	Fipronil 10 % w/v	Methoprene 12 % w/v	Fipronil (10% w/v) + Methoprene (12% w/v)
Pretreatment	34.8	28.3	36.5	36.9
Day 1	50.6	-	0	-
% Reduction		-	100	-
Day 22	39.8	-	0	-
% Reduction		-	100	-
Day 29	42.6	0 [C]	0.3	0 [D]
% Reduction		100	99.4	100
Day 36	34.6	42.5 [E]	5.2	1.4 [F]
% Reduction		0	85.0	95.9
Day 43	55.9	58.6	4.6	3.6
% Reduction		0	91.7	93.6
Day 50	39.2	54.9	18.9	8.7
% Reduction		0	51.6	77.9
Day 57	53.8	46.0	50.4	27.3
% Reduction		14.4	6.3	49.2

[A] Retransformed mean of radians; based on the transformation are sine √(number of adults/number of eggs).
[B] Eggs were collected for 24 hours starting 72 hours after infestation. Approximately 100 eggs were incubated for 35 days.
[C] One animal with a few eggs; no adults developed.
[D] Three animals with eggs; no adults developed.
[E] Five animals with eggs incubated.
[F] Six animals with eggs incubated

The results of these trials are depicted in Fig. 1 and Fig. 2 respectively.

As is apparent from the data, the inventive compositions comprising fipronil and (S)-methoprene are surprisingly more ovicidally active for an extended time than composition comprising just fipronil or (S)-methoprene alone.

B. Ovicidal Inhibition Tests in Dogs

This test determines the effect that a flea composition has on the inhibition of larvae hatching from fleas eggs and on the inhibition of adult cat fleas emergence when the composition is applied to the skin of dogs infested with newly emerged adult cat fleas (*Ctenocephalides felis*). Thirty-six (8 female and 24 male) Beagle dogs approximately 12.7-65.9

months old and weighing 10.2-19.8 kg were selected and housed in individual runs. On Day –12 each dog was infested with approximately 200 adult cat fleas. One Day –11 the dogs were combed to remove and count the fleas and they were re-infested with approximately 200 adult fleas. At approximately 72 hours post-infestation, a procedure for collection of flea eggs was begun. Eggs were collected over approximately a 24-hour period. On Day –7 two aliquots of approximately 100 eggs each were formed from the eggs collected form each animal. One of these aliquots was incubated at approximately 23-30° C and 70-90% RH for approximately 72 hours to determine larval hatch. The other aliquot was incubated under the same conditions for 35 days to determine the number of adult fleas that developed. Eight replicates of four animals were formed (four dogs were dropped from the trial) based on body weight within sex. One dog in each replicate was randomly allocated to each of four treatment groups 1) untreated control; 2) fipronil 10% w/v solution; 3) (S)-methoprene 9% w/v solution; and 4) fipronil 10% w/v and (S)-methoprene 9% w/v combination solution. Treatments of the flea composition were applied once topically on Day 0 at the rate 0.067 ml/kg body weight. On days 1, 22 and weekly to Day 85 each dog was infested with approximately 200 adult fleas. Eggs were collected over approximately 100 eggs, if available, from each animal at each infestation time was incubated for three days to determine larval hatch and the other aliquot incubated for 35 days to determine the number of adults that developed. The results of this trial are reported in Table 9 (Dog Dose Confirmation Trial – Percentage of Larvae That Hatch) and Table 10 (Dog Dose Confirmation Trial – Percentage of Adult Fleas That Develop).

TABLE 9
Methoprene Dose Trial in Dogs Percentage [A] of Larvae that Hatch

Infestation Day [B]	Untreated Control	Fipronil 10 % w/v	Methoprene 9 % w/v	Fipronil (10% w/v) + Methoprene (9% w/v)
Pretreatment	74.9	71.9	71.0	68.4
Day 1	77.6	-	0	-
% Reduction		-	100	-
Day 22	76.4	-	0.1	-
% Reduction		-	99.8	-
Day 29	75.0	-	0.8	-
% Reduction		-	98.9	-
Day 36	63.7	22.2*	2.7	-
% Reduction		65.1*	95.8	-
Day 43	78.8	51.6**	5.3	1.8***
% Reduction		34.5**	93.9	97.8***
Day 50	75.1	49.4	6.0	6.8
% Reduction		34.3	92.0	90.9
Day 57	76.8	56.2	29.7	1.4
% Reduction		26.8	61.3	98.2
Day 64	78.0	59.2	30.0	8.5
% Reduction		24.2	61.5	89.1
Day 71	75.3	62.7	30.6	8.1
% Reduction		16.7	59.3	89.3
Day 78	85.3	49.4	30.6	12.4
% Reduction		42.1	64.1	85.5
Day 85	76.1	61.8	50.4	26.9
% Reduction		18.8	33.7	64.6

[A] Retransformed mean of radians; based on the transformation are sine √(number of larvae/number of eggs).
[B] Eggs were collected for 24 hours starting 72 hours after infestation. Approximately 100 eggs were incubated for 72 hours.
* One animal with 36 eggs incubated; 8 larvae hatched (22.2%).
** Six animals with eggs incubated.
*** Three animals with eggs incubated.

TABLE 10
Methoprene Confirmation Trial in Dogs Percentage [A] of Adults that Develop

Infestation Day [B]	Untreated Control	Fipronil 10 % w/v	Methoprene 9 % w/v	Fipronil (10% w/v) + Methoprene (9% w/v)
Pretreatment	55.3	58.0	57.5	56.1
Day 1	56.5	-	0	-
% Reduction		-	100	-
Day 22	57.4	-	0.1	-
% Reduction		-	99.8	-
Day 29	55.5	-	0.8	-
% Reduction		-	98.5	-
Day 36	56.9	-*	0.5	-
% Reduction		-*	99.2	-
Day 43	59.2	20.5**	2.5	0.7***
% Reduction		65.4**	95.8	98.8***
Day 50	53.5	23.2	4.7	2.2
% Reduction		56.7	91.3	95.9
Day 57	57.4	38.8	13.8	0.3
% Reduction		32.5	75.9	99.4
Day 64	55.5	39.2	18.9	3.8
% Reduction		29.5	66.1	93.1
Day 71	53.5	45.7	13.9	2.2
% Reduction		14.6	74.0	95.9
Day 78				
% Reduction				
Day 85				
% Reduction				

[A] Retransformed mean of radians; based on the transformation arc sine √(number of adults/number of eggs).
[B] Eggs were collected for 24 hours starting 72 hours after infestation. Approximately 100 eggs were incubated for 35 days.
* One animal with 35 eggs incubated; 4 adults developed (11.4%).
** Six animals with eggs incubated. *** Three animals with eggs incubated.

The results of these two trials are depicted in Fig. 3 and Fig. 4 respectively.

As is apparent from the data, the inventive composition comprising fipronil and methoprene are surprisingly more ovicidally active for an extended period of time than compositions comprising just fipronil or (S)-methoprene alone.

9. Based upon the data presented above, I conclude that the inventive composition comprising a combination of fipronil and (S)-methoprene exhibit surprisingly superior ovicidal activity in inhibiting larvae from hatching and for extended periods of time. As one would not expect this in view of the state of the art this activity is unexpected. Moreover, from this observation one would expect that the inventive compositions would have economic

importance in the marketplace in view of their long duration. Further, in view of the fact that insect growth possesses similar activities, one could also conclude that such results would be obtained with other compounds which exert their activity by mimicking the juvenile hormone.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further, that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: September 30, 1999

By: Alan Marchiondo
Alan Marchiondo, Ph.D.

Fig. 1

- % Reduction in Proportion of Larvae that Hatched by Treatment and Day of Flea Challenge (Eight Cats per Treatment)

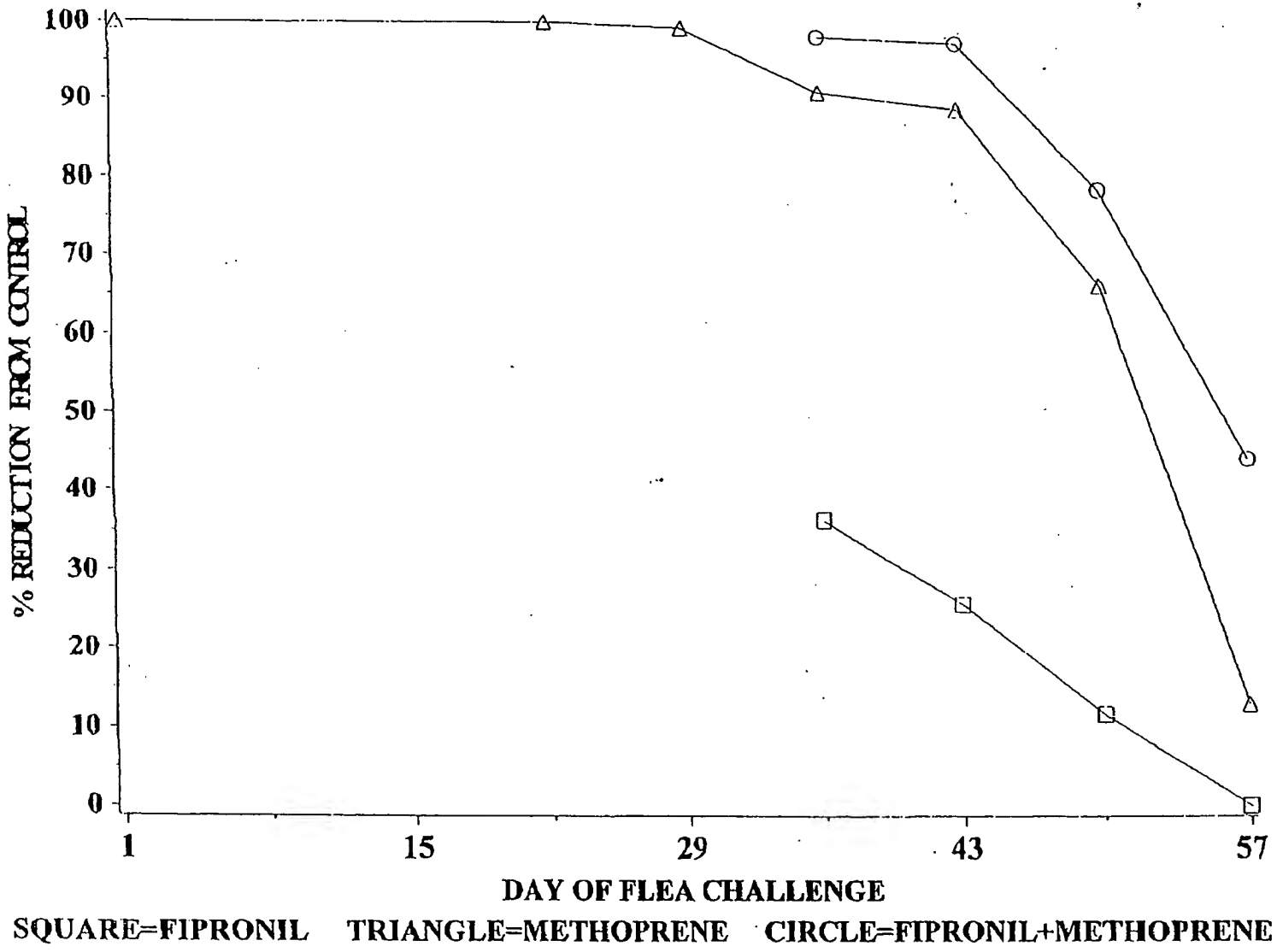


Fig. 2 - % Reduction in Proportion of Adults that Developed by Treatment and Day of Flea Challenge (Eight Cats per Treatment)

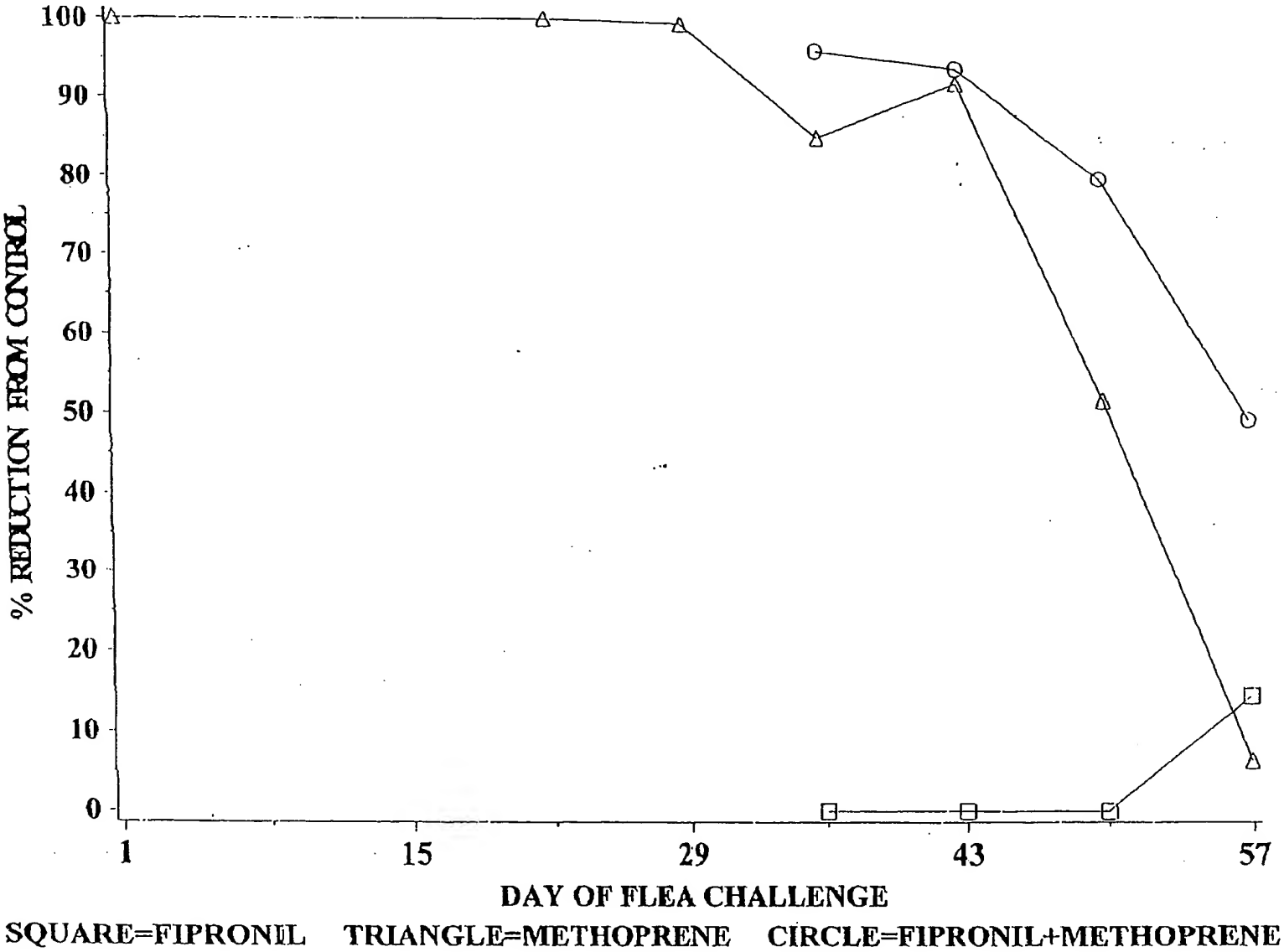
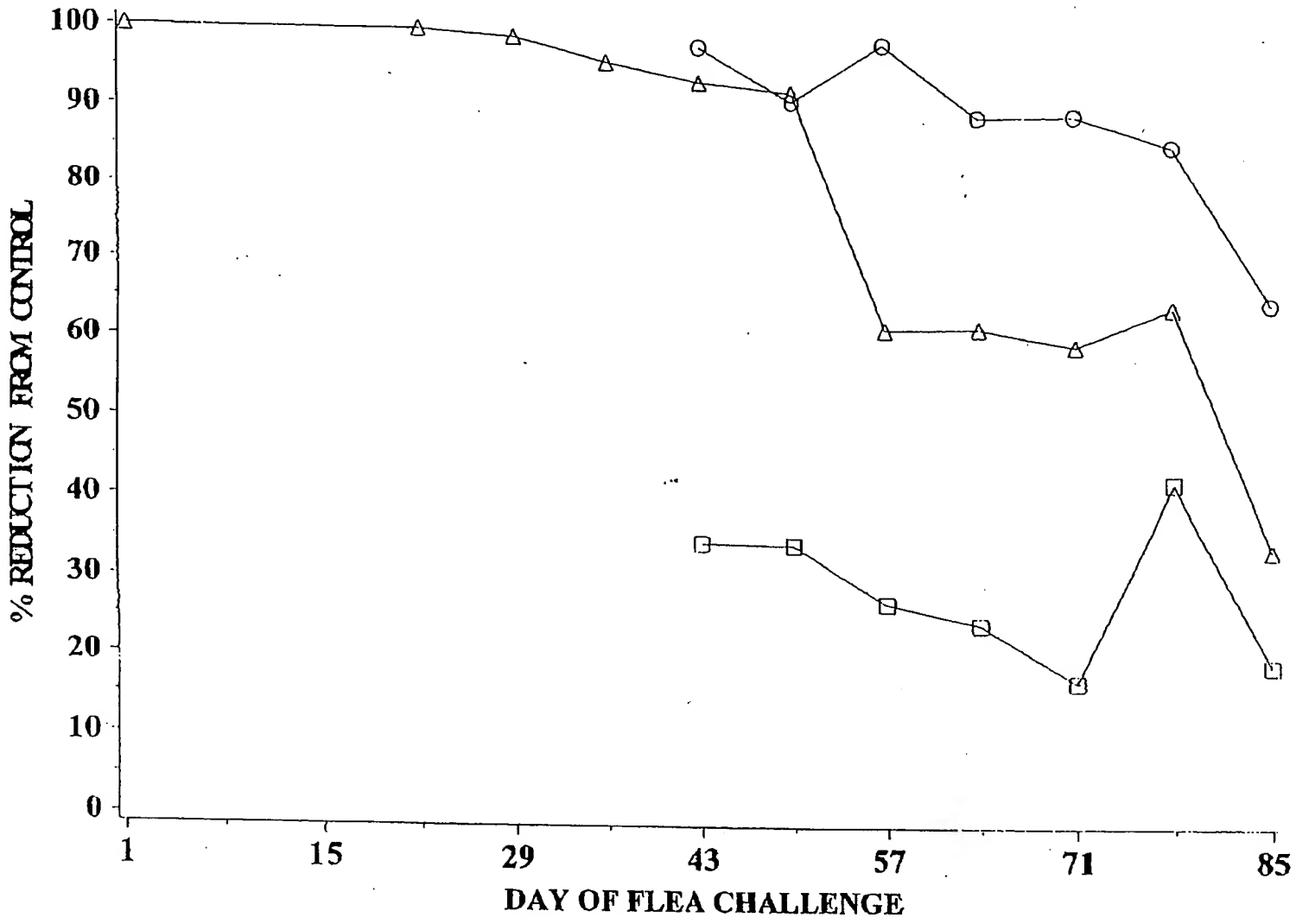
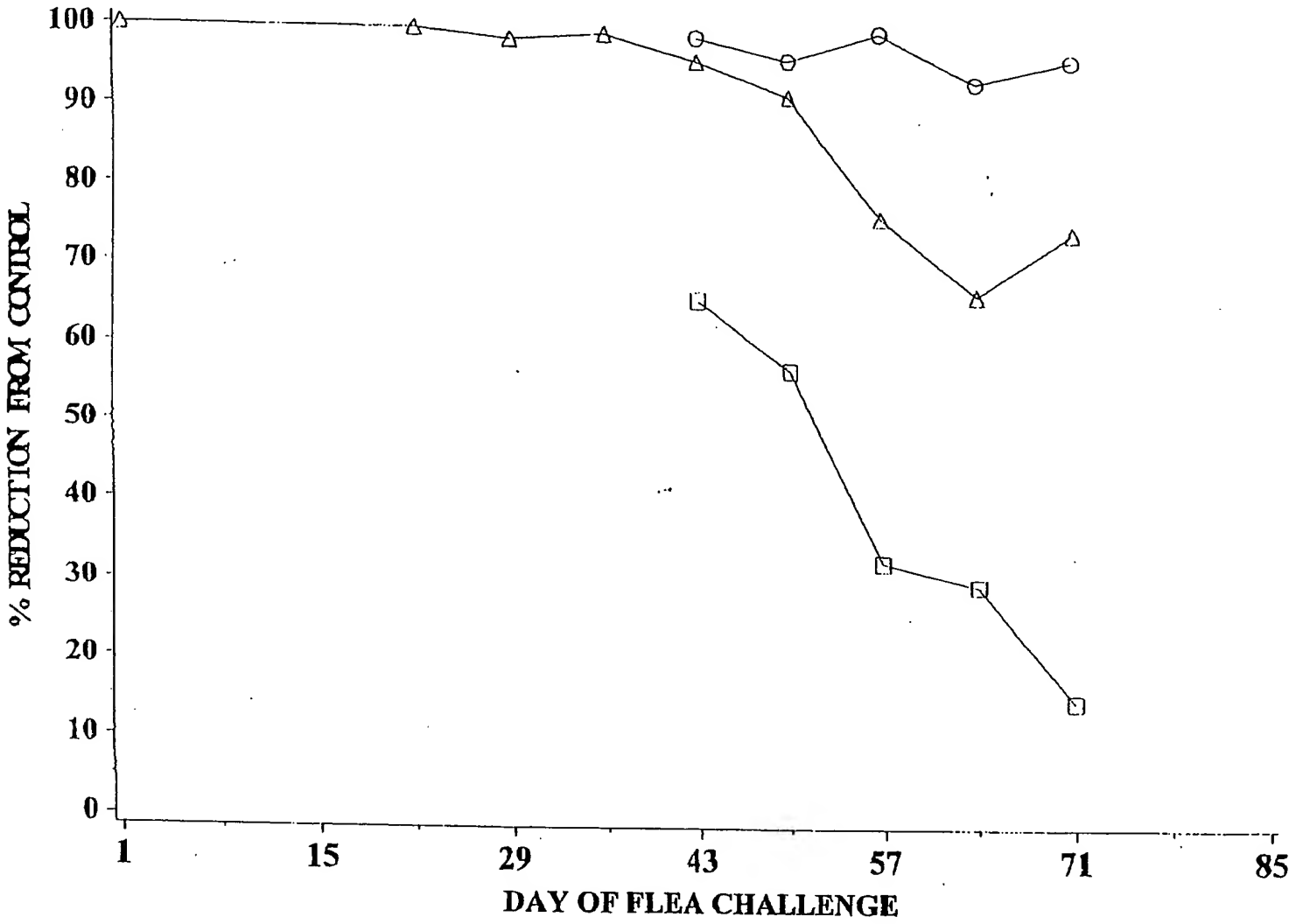


Fig. 3 - % Reduction in Proportion of Larvae that Hatched by Treatment and Day of Flea Challenge (Eight Dogs per Treatment)



SQUARE=FIPRONIL TRIANGLE=METHOPRENE CIRCLE=FIPRONIL+METHOPRENE

Fig. 4 - % Reduction in Proportion of Adults that Developed by Treatment and Day of Flea Challenge (Eight Dogs per Treatment)



92000

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EDUCATION

NIH Post-Doctoral Fellowship, 1984 (Parasitology)
University of Notre Dame, South Bend, IN.
Basic research in helminth physiology and developmental biology of nematodes and cestodes.

Ph.D., Zoology, 1982 (Parasitology)
Brigham Young University, Provo, UT.
Dissertation Title: Comparative Biological Aspects of Taeniidae with Special Reference to *Echinococcus*: Chemotherapy, Speciation, Elemental Analysis, and Ultrastructural Observations.

M.S., Biology, 1974 (Parasitology)
University of New Mexico, Albuquerque, NM.
Thesis Title: *Toxoplasma* Antibodies in Wild and Domiciled Animals from Arizona, Colorado, Montana, New Mexico, and the Philippines.

B.S., Biology, 1971
University of New Mexico, Albuquerque, NM.

PROFESSIONAL BACKGROUND

MERIAL LIMITED, MISSOURI RESEARCH CENTER, FULTON, MO (Position Transfer Result of Merger of Animal Health Divisions of Merck & Co. and Rhone Poulenc S.A.)

Research Scientist 1/98-Present.
Conduct pharmaceutical and biological evaluations (basic, preclinical or clinical) and target animal safety trials for developmental research and registration of animal health products in regional and global markets. Supervise maintenance and propagation of ecto- and endoparasite colonies/cultures for in vitro screening and *in vivo* efficacy trials.

RHONE MERIEUX, INC., RHONE POULENC AG CO., RESEARCH TRIANGLE PARK, NC

Manager of Biology/Project Manager, Parasiticide Evaluation 11/95-12/97.
Directly responsible for management of the Rhone Merieux parasiticide discovery research program at Rhone Poulenc Ag including primary (conventional and high throughput), secondary and tertiary screening and pre-clinical development for pet, companion and food producing animals.

FERMENTA ANIMAL HEALTH COMPANY, KANSAS CITY, MO

Director, Pet Insecticides & Anthelmintics, 1/92-10/95.
Directly responsibility for domestic and international new product research, commercial development and regulatory approval of small animal and household insecticides and endoparasiticide products for pet, companion and food producing animals.

Manager, Parasiticide Development, 2/89-12/91.
Directly responsible for commercial development of ectoparasiticide products containing insect growth regulators (IGRs) for premise and on-animal uses and endoparasiticide products for pets, swine and cattle.

Product Development Parasitologist 5/87-1/89.
Responsible for new product research and commercial development of potential parasiticides for domestic and international animal health markets.

SDS BIOTECH CORP./RICERCA. INC., PAINESVILLE, OH

Senior Research Parasitologist, 8/84-4/87.
Direct responsibilities included supervision of primary screening, secondary evaluations and new product research of potential parasiticides as well as provided research support of existing parasiticide products.

MOST SIGNIFICANT ACCOMPLISHMENTS

Basic Research

Identified a new chemical class of anthelmintic, a systemic flea and tick adulticide for pets, and a new phenylpyrazole compound highly active against ectoparasites of food producing animals.

Successfully incorporated in vitro parasiticide targets into the high throughput screening program at Rhone Poulenc Ag Co.

Established *in vivo* parasiticide screening programs at various research facilities throughout the U.S.A. and in Brazil.

Discovered the selective toxicity of diazinon against pyrethroid-resistant horn flies. This discovery lead to the commercial development of the first insecticidal cattle ear tag (Terminator®, Fermenta Animal Health Co.) and subsequent diazinon ear tags (Patriot®, Fermenta Animal Health Co.) effective against pyrethroid-resistant horn flies, 1985.

Identified and described the biological and morphological modes of action of the IGR, fenoxycarb (Tenocide™), against the life cycle stages of the cat flea, 1990.

Systemic flea ovicidal efficacy of fenoxycarb demonstrated via oral and percutaneous routes of administration, 1990.

Developed a method of preparing permanent light microscopy slides of coccidian oocysts. No significant changes occurred in the morphology of the oocysts for over 10 years, 1978-1988.

Product Development

Commercially developed and registered eight domestic and three international insecticide products for flea and tick control. Highly experienced with topical and systemic insect growth regulators and adulticidal compounds.

Development experience with several small and large animal generic anthelmintics (tablets, granules, suspensions, pour-ons and injectables) and have conducted *in vivo* blood-level and clinical end-point bioequivalency evaluations.

Experienced in flea and tick collar development as well as pet shampoos, topical spot-on and pour-on formulations, aerosol carpet sprays, foggers and lawn sprays.

Expanded federal EPA label of Rabon® Oral Larvicide by establishing suspendability and manufacturing guidelines for use in bovine liquid feed supplements.

Developed strategic deworming program for swine with Atgard© C.

Transdermal diethylcarbamazine product developed for monthly prophylaxis of canine dirofilariasis.

PROFESSIONAL MEMBERSHIPS

American Association for the Advancement of Science, 1984-Present.

American Society of Parasitologists, 1973-Present. Various offices held.

American Association of Veterinary Parasitologists, 1984-Present. Various offices held.

World Association for the Advancement of Veterinary Parasitology, 1984-Present.

RESEARCH-RELATED SERVICE

Editorial Board Member - **Veterinary Parasitology**, 1988-Present.

Peer Reviewer: **Journal of Parasitology**, 1986-Present

Parasitology Research, 1990-Present.

Journal of Agriculture, Ecosystems & Environment, 1994-Present.

Journal of Aquatic Animal Health, 1996-Present.

SPECIALIZED TRAINING

Molecular Biochemistry and Physiology of Helminth Neuromuscular Systems/Molecular Basis of Drug Design and Resistance, City University, London, U.K., 1996.

FDA Workshop on Compliance Requirements for Animal Drug Manufacturers: General Principles of Process Validation, Kansas City, MO, 1991.

FDA Workshop on Compliance Requirements for Animal Drug Manufacturers:Good Manufacturing Practices, Kansas City, MO, 1990.

Molecular Paradigms for Eradicating Helminth Parasites. UCLA Symposia on Molecular & Cellular Biology, Steamboat Springs, CO, 1987.

The Johns Jacob Abel Symposium on Drug Development. The Johns Hopkins University School of Medicine, Baltimore, MD, 1983.

SCIENTIFIC PUBLICATIONS

Thirty-six publications in refereed journals. Selected publications are listed below.

Marchiondo, A.A. and F.L. Andersen. 1985. *In vivo* efficacy and ultrastructural effects of mitomycin C against experimental alveolar hydatid disease. J. Helminthol. 59:29 38.

Marchiondo, A.A. and J. Szanto. 1987. Efficacy of dichlorvos, fenbendazole, and ivermectin in swine with induced intestinal nematode infections. Am. J. Vet. Res. 48:1233-1235.

Sheppard, D.C. and A.A. Marchiondo. 1987. Toxicity of diazinon to pyrethroid resistant and susceptible horn flies, *Haemaobia irritans* (L.): Laboratory studies and field trials. J. Agric. Entomol. 4:262-270.

Kennedy, T.J., Bruer, D.J., A.A. Marchiondo, and J.A. Williams. 1988. Prevalence of swine parasites in major hog producing areas of the United States. Agri-Practice. 9:25-32.

Marchiondo, A A., P.P. Weinstein, and J.F. Mueller. 1989. Significance of the distribution of ^{57}Co-vitamin B_{12} in *Spirometra mansonoides* (Cestoidea) during growth and differentiation in mammalian intermediate and definitive hosts. Int. J. Parasitol. 19:119-124.

Marchiondo, A.A., J.L. Riner, D.E. Sonenshine, K.F. Rowe and J.H. Slusser. 1990. Ovicidal and larvicidal modes of action of fenoxycarb against the cat flea (Siphonaptera: Pulicidae). J. Med. Entomol. 27: 913-921.

Marchiondo, A.A., R. Ming, F.L. Andersen, J.H. Slusser and G.A. Conder. 1994. Enhanced larval cyst growth of *Echinococcus multilocularis* in praziquantel-treated jirds, *Meriones unguiculatus*. Am. J. Trop. Med. Hyg. 50:120-127.

Marchiondo, A.A., S. M. Meola,. K.G. Palma, J.H. Slusser and R.W. Meola. 1999. Chorion formation and ultrastructure of the egg of the cat flea (Siphonaptera, Pulicidae). J. Med. Entomol. 36 (2): 149-157.

Wall, R., K.E. Smith, J.J. Howard, L. Strong, A.A. Marchiondo and P. Jeannin. 1999. In vitro insecticidal effects of the phenylpyrazole, Fipronil, and the synthetic pyrethroid, β-Cyfluthrin, on larvae of the sheep blowfly, *Lucilia sericata*. Vet. Parasitol. In Review.

NON-REFEREED JOURNAL PUBLICATIONS

Szanto, J. and A.A. Marchiondo. 1986. Whipworm infection in swine. Animal Health & Nutrition. 41:18-22.

Marchiondo, A.A. 1987. Biology, economic effect and control of the horn fly. Animal Health & Nutrition. 42:6-10. Reprinted in Norden News, 1987, 62:10-16. Reprinted in CHIKUSAN-NO-KENKYU {Animal Husbandry) in Japanese, 1994, 48(5):69-73.

Marchiondo, A.A. and J.L. Riner. 1988. Integrated control program for the horn fly. Large Animal Vet. 43:22-24.

Marchiondo, A.A., J.L. Riner and H.R Sinclair. 1989. Mode of action of insect growth regulators against fleas. Proceedings of EXPO 1989, Veterinary Learning Systems, pp. 25-26.

Marchiondo, A.A. 1993. Safe and effective flea control for cats. Veterinary Technician 14(4):235-245.

Marchiondo, A.A. and G.A. Conder. 1994. A correlative LM/SEM method. USA Microscopy and Analysis, July, Issue 7, p. 11.

PAPERS PRESENTED AT SCIENTIFIC MEETINGS

Over 25 presentations given since 1977. Selected presentations are listed below.

Andersen, F.L., A.A. Marchiondo, and G.A. Conder. 1981. Efficacy of praziquantel against *Echinococcus* spp. 26th Annual Meeting of the American Association of Veterinary Parasitologists, St. Louis, MO.

Marchiondo, A.A. 1986. Comparative efficacy and economic performance of Atgard C and Ivomec against *Ascaris suum*, *Trichuris suis*, and *Oesophagostomum dentatum* in experimentally-infected swine. 104th Annual Meeting of the Iowa Veterinary Medical Association, Des Moines, IA and the American Association of Swine Practioners, Minneapolis, MN.

Marchiondo, A.A. and D.C. Sheppard. 1987. Toxicity of diazinon to pyrethroid-resistant horn flies: Laboratory studies and field trials. 31st Annual Livestock Insect Workers Conference, Beltsville, MD.

Marchiondo, A.A., J.L. Riner, D.E. Sonenshine, K.F. Rowe and J.H. Slusser. 1989. Modes of action of fenoxycarb against developmental stages of the cat flea, *Ctenocephalides felis*. 64th Annual Meeting of the American Society of Parasitologist, Vancouver, BC.

Marchiondo, A.A., S. Ackers, S.W. Fogt, D.L. Heimbichner and R. Young. 1992 & 1993. Efficacy and safety of fenoxycarb pet spray for control of *Ctenocephalides felis* infestations on dogs and cats. 37th Annual Meeting of the American Association of Veterinary Parasitologists, Boston, MA, and Second International Symposium on Ectoparasites of Pets, Lexington, KY.

Marchiondo, A.A., S.M. Meola, R.W. Meola, K.G. Palma and J.H. Slusser. 1995. Ultrastructure of the egg of the cat flea. Third International Symposium on Ectoparasites of Pets, College Station, TX.

Marchiondo, A.A., S.W. Fogt, D.L. Heimbichner and S.L. Stroh. 1995. Efficacy of azadirachtin and pyriproxyfen against developing stages of the cat flea, *Ctenocephalides felis*. Joint Meeting of the American Society of Parasitologists & the American Association of Veterinary Parasitologists, Pittsburgh, PA.

Marchiondo, A.A., S.M. Meola, K.G. Palma, R.W. Meola and J.H. Slusser. 1996. Development and ultrastructure of the cat flea eggshell. Joint Meeting of the American Society of Parasitologists & the Society of Protozoologists, Tucson, AZ.

Gant, D.B., A.B. Chalmers, M.A. Wolff, H.B. Hoffman and D.F. Bushey. 1996. Mode of action of Fipronil. Presented by A.A. Marchiondo. 41st Annual Meeting of the American Association of Veterinary Parasitologists, Louisville, KY.

Dryden, M.W., A.A. Marchiondo, S. Ackers and S.W. Fogt. 1996. Residual control of *Ctenocephalides felis* development by single site applications of insect growth regulators on infested cats. 41st Annual Meeting of the American Association of Veterinary Parasitologists, Louisville, KY.

McCall, J.W., S.L. Stroh, A.A. Marchiondo and T.L. McTier. 1997. Canine heartworm prophylaxis by high oral dosages or transdermal administration of Diethylcarbamazine. 42nd Annual Meeting of the American Association of Veterinary Parasitologists, Reno, NV.

Marchiondo, A.A., S.E. Green, R.E. Plue, D.H. Wallacae, R.A. Barrick and P. Jeannin. 1999. Comparative speed of kill of Frontline® Top-Spot™, Frontline® Spray and Advantage® against adult cat fleas (*Ctenocephalides felis*) on dogs. 5th International Symposium on Ectoparasites of Pets, Fort Collins, CO.

Marchiondo, A.A., C. Robertson-Plouch, R.A. Barrick, J. Guerrero, and P. Jeannin. 1999. Comparative speed of kill of Frontline® Top-Spot™, Frontline® Spray and Advantage® against adult cat fleas (*Ctenocephalides felis*) on dogs. 5th European Small Animal Veterinary Congress : FECAVA, Lyon, France.